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Prevalence of *Borrelia burgdorferi* and *Borrelia miyamotoi* in questing *Ixodes ricinus* ticks from four sites in the UK

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ABSTRACT

Borrelia miyamotoi is a spirochete bacterium related to *Borrelia burgdorferi sensu lato*, the cause of Lyme borreliosis, and vectored by ticks. In 2014, *B. miyamotoi* was identified in three questing *Ixodes ricinus* collected in the UK. We sought to confirm the presence of *B. miyamotoi* in the UK. Ticks were collected from four locations not previously investigated for *B. miyamotoi* or *B. burgdorferi s.l* and of which two are considered as Lyme borreliosis "hotspots" based on hospital records of the disease. We independently confirm that *B. miyamotoi* is present in the UK and support the view that *B. miyamotoi* is likely to have a broad geographic distribution, at low levels. Our study also adds to the existing data on the distribution of *B. burgdorferi s.l* in the UK and demonstrates that although the two "hotspots" had relatively high tick densities, they did not have the highest proportion of infected ticks.

Introduction

Ticks transmit various pathogens that cause zoonoses, one of which is Lyme borreliosis, caused by the spirochete *Borrelia burgdorferi sensu lato*. In Europe, the hard tick, *Ixodes ricinus* is the most common vector of this tick-borne bacterium. The *B. burgdorferi s.l.* complex comprises a number of genospecies, the most important ones in the UK being *B. burgdorferi sensu stricto* (*B. burgdorferi s.s.*), *B. garinii* and *B. afzelii*. In humans, these commonly cause erythema migrans, fever, arthralgia, myalgia and fatigue (Dubrey et al., 2014). The number of recorded cases of Lyme borreliosis in England and Wales has been steadily rising from 268 in 2001 to 959 in 2011, an increase of 258% (Public Health England, 2013). There are several possible reasons which have been put forward to explain this increase such as the increased sensitivity of diagnostic tests, the increased availability of diagnostic services and greater awareness of Lyme borreliosis symptoms (Public Health England, 2013). It has also been suggested that tick populations have increased as a result of increases in deer populations (Scharlemann

et al., 2008), however deer can clear *B. burgdorferi s.l.* from ticks and therefore this may not be responsible for the increase in Lyme borreliosis (Roome et al., 2017). Up to 2000 cases annually are thought to go undiagnosed (British Infection Association, 2011).

Several regions in the UK have been classed as tick and Lyme borreliosis "hotspots" based on areas understood to have a high tick density and where many cases of the disease have been reported. These include Exmoor, the Lake District, Thetford, New Forest, Salisbury Plain, the South Downs, West Sussex, Surrey, West Berkshire, Wiltshire, Yorkshire moors, Scottish Highlands, Richmond Park (London) and Bushy Park (London) (Dubrey et al., 2014). Although the tick infection prevalence in questing ticks in some of these hotspots has been examined (Davidson et al., 1999, Kurtenbach et al., 2001, Vollmer et al., 2011, James et al., 2013, Hansford et al., 2015), others have not been investigated. Data on questing tick density and tick infection prevalence are necessary to identify which areas pose most risk to individuals, rather than identifying these areas based on the number of Lyme borreliosis cases, which is dependent on visitor numbers.

A species related to *B. burgdorferi s.l.*, known as *Borrelia miyamotoi*, has been reported to cause illness similar to Lyme borreliosis (Platonov et al., 2011). *B. miyamotoi* was first recorded in Japanese *Ixodes* ticks in 1995 (Fukunaga et al., 1995) and has since been found in ticks collected in Russia (Platonov et al., 2011), the USA (Ullmann et al., 2005, Hamer et al., 2014), Canada (Ogden et al., 2011, Dibernardo et al., 2014) and parts of Europe (Wilhelmsson et al., 2010). In immunocompetent individuals, *B. miyamotoi* causes influenza-like symptoms including relapsing fever, headache, nausea, fatigue, myalgia and arthralgia (Branda and Rosenberg, 2013). Although *B. miyamotoi* infection is frequently referred to as a relapsing fever, it has been argued that it should be termed "*Borrelia miyamotoi* disease" since the symptoms are less severe than a tick-borne relapsing fever (Telford III et al., 2015). In immunocompromised individuals, more severe symptoms such as meningoencephalitis have been noted (Gugliotta et al., 2013, Hovius et al., 2013). In 2014, *B. miyamotoi* was recorded in three nymphal *I. ricinus* ticks collected in the UK (Hansford et al., 2015) but this observation has yet to be independently confirmed. Human infections caused by *B. miyamotoi* in the UK have not been reported or identified so understanding the geographical prevalence of this organism in UK ticks can assist in assessing risk and likelihood of infection.

Besides investigating if *B. miyamotoi* is present in questing ticks in the UK, the aims of this study were to compare the tick density and prevalence of tick infection with *B. burgdorferi s.l.* and *B. miyamotoi* between two tick and Lyme borreliosis "hotspots" (Thetford Forest and West Dean), a location understood to have a high tick density (Cirencester Park), and a location where *I. ricinus* have not established despite a favourable habitat (Wytham Woods). These locations were chosen because they have not been investigated previously and they are separated spatially by at least 45km, covering different regions of England. Questing ticks were collected by blanket dragging at each of the four locations during spring 2014 and screened for *Borrelia* by multiplex quantitative PCR. *Borrelia* genospecies of positive samples were identified by DNA sequencing.

Materials & Methods

Tick Collection

Ticks were collected from 4 locations in the United Kingdom (Fig. 1): Cirencester Park, Gloucestershire (51.713163,-2.060698) on 18th March 2014; West Dean, South Downs National Park, West Sussex (50.939146,-0.786328) on 26th March 2014; Kings Forest, Thetford Forest, Suffolk (52.351393,0.676978) on 9th April 2014, and Wytham Woods, Oxfordshire (51.774881,-1.331728) on 14th April 2014. Weather conditions were dry with temperatures between 8-18°C. At each location, 2 woodland habitat sites and 2 ecotone habitat sites adjacent to woodland were surveyed using the blanket dragging method (Macleod, 1932). For Thetford and West Dean, one woodland site was deciduous and the other evergreen whereas for Cirencester and Wytham Woods both woodland sites were deciduous.

The blanket was 1.5 metres wide and 1.85 metres long (2.775m^2). It was dragged 6m and then checked for ticks. Any ticks found were placed inside a 1.5ml Eppendorf tube. This was repeated 10 times at each site resulting in 60m total distance dragged at each site. However, for Cirencester, 5 sites were sampled of which 3 were ecotone and 2 were woodland. The first site (A) was dragged in 20 x 6m repeats resulting in 120m of habitat sampled. For Site B there were 5 x 6m repeats. Ticks were stored at -80°C until DNA extraction.

DNA extraction

DNA for PCR analysis was extracted from the ticks, using an ammonium hydroxide lysis method, adapted from Guy and Farquhar, 1991. The ticks were placed individually into the wells of a 96 well plate. 100 µl of 1M ammonium hydroxide (Sigma Aldrich) was pipetted into each well. Negative extraction controls were incorporated into the extraction process. The plate was sealed and placed in a PCR thermal cycler at 99°C for 20 minutes to lyse the samples. After lysis, the plate was briefly centrifuged at 1000rpm and the plate seal was removed. The plate was then incubated at 99°C for a further 20 minutes to evaporate the ammonia. Approximately 50µl of solution remained. The samples were stored at -20°C until required.

Detection of *Borrelia burgdorferi sensu lato* and *Borrelia miyamotoi*

Multiplex qPCR was used to determine which tick extracts contained *B. burgdorferi s.l.* and *B. miyamotoi* according to the method by Hansford et al., 2015, refer to this paper for primer and probe sequences. Each well of the PCR plate contained 20 µl comprising 400 nM B-OspA_modF primer, 400 nM B-OspA_borAS primer, 100 nM B-OspA_mod-probe, 200 nM B-FlaB-F primer, 100 nM B-FlaB-Rc primer, 100 nM B-FlaB-Rt primer, 200 nM B-FlaB-FAM probe, 200 nM FlabBm.motoiF primer, 200 nM FlabB.m.motoiR primer, 200 nM FlabBm.motoiP primer, 10 µl 2x iQ multiplex Powermix (Bio-rad) and 5 µl sample. Molecular grade water was used as a negative control and *B. burgdorferi s.s* DNA as a positive control. The PCR was performed using the Applied Biosystems 7500 Fast Real-time PCR Machine. The program consisted of 5 minutes at 95°C followed by 45 cycles of 5 seconds at 94°C and 35 seconds at 60°C.

Differentiating between *Borrelia* species

Sequencing 5S-23S intergenic spacer

The sequence of the 5S-23S intergenic spacer for each *B. burgdorferi s.l* -positive sample was determined for comparison with the results of the *recA* typing assay. Touch down PCR was first used to amplify the intergenic region in the positive samples. The primers B5Sborseq and 23Sborseq are used as in the method described by Heylen et al., 2013. Each reaction contained 5 µl 10x Platinum Taq buffer (without $MgCl_2$), 1 µl 10mM dNTPs, 3 µl 25mM $MgCl_2$, 2 µl 10µM B5Sborseq, 2 µl 10µM 23Sborseq, 0.2 µl Platinum Taq (Invitrogen), 31.8 µl H_2O and 5 µl sample. The procedure included H_2O as a negative control and *B. afzelii*, *B. garinii* and *B. burgdroferi s.s.* DNA as positive controls. The PCR was performed using an Applied Biosystems Veriti® Thermal Cycler. The PCR program consisted of a Taq activation step at 94°C for 5 minutes followed by 94°C for 20 seconds (denaturation), 70°C for 30 seconds (annealing) and 72°C for 30 seconds (elongation) for 10 cycles, lowering by 1°C per cycle. The program continued for 40 cycles at: 94°C for 20 seconds, 60°C for 30 seconds and 72°C for 30 seconds. There was then a final extension step at 72°C for 7 minutes.

The products were then analysed by nucleic acid electrophoresis on a 2% TBE agarose gel. Products were sequenced on a 3130xL Genetic Analyzer using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The computer program SeqTrace (Stucky, 2012) was used to create the consensus sequences from the forward and reverse primer sequences.

Genospecies identification

The *Borrelia* genospecies to which the sequences belong were found by inputting the sequences into NCBI's BLAST program (Altschul et al., 1990) and locating the best match with the lowest E value through multiple alignment against reference sequences.

Statistics

Tick densities and the percentage of ticks with *B. burgdorferi s.l.* across the locations were examined by Two-way ANOVA using R Project (R Core Team, 2015). The chi-square test was used to compare the proportion of ticks infected with *B. burgdorferi s.l.* and *B. miyamotoi* between life cycle stage, habitat and location. The proportions of *B. burgdorferi* genospecies of infected ticks between locations were compared using Fisher's Exact Test. The significance level used was $P<0.05$. These statistical tests were performed in JMP 11.1.1 (SAS Institute Inc.).

Results

Tick Collection

Ticks were found in all of the sites sampled. In total, 832 ticks were collected comprising 766 nymphs, 37 adult males and 29 adult females. Similar numbers of ticks were collected from each location over a similar period of time. The exception was Wytham Woods where only 11 ticks were collected; however, this was the first record of questing *I. ricinus* in Wytham Woods. Adult ticks were collected from all but 3 of the sites sampled across all locations. For comparison between sites, the data were converted to the number of ticks per $100m^2$ sampled (Table 1).

Mean nymph density differed significantly between locations (Two-way ANOVA, $F_{3,9}=5.209$, $P=0.0233$) and was significantly greater in ecotone habitats than woodland habitats (Two-way ANOVA, $F_{1,9}=5.183$, $P=0.0488$) (Fig. 2a.). The effect of habitat type on nymph density did not depend on the location (Two-way ANOVA, $F_{3,9}=0.880$, $P=0.4873$). Mean adult tick density also differed significantly between locations (Two-way ANOVA, $F_{3,9}=17.687$, $P=0.0004$) (Fig. 2b.). Thetford Forest had a significantly higher mean adult tick density than West Dean, Cirencester and Wytham Woods (Tukey Kramer HSD Test, $P=0.0376$, $P=0.0045$ and $P=0.0003$, respectively). Mean adult density was significantly greater in ecotone habitats than woodland habitats (Two-way ANOVA, $F_{1,9}=28.713$, $P=0.0005$) (Fig. 2b.). The effect of habitat type on tick density did not depend on the location (Two-way ANOVA, $F_{3,9}=0.182$, $P=0.9061$).

Detection of *Borrelia burgdorferi sensu lato* and *Borrelia miyamotoi*

Of the 832 ticks collected, 825 ticks were analysed (7 samples from West Dean were lost during ammonium hydroxide extraction). Multiplex qPCRs were used to detect samples containing *B. burgdorferi s.l* and/or *B. miyamotoi* (Table 2). In total, 26 ticks (25 of which were nymphs) were PCR positive for *B. burgdorferi s.l.* (3.15%, 95% confidence interval (CI) 2.16-4.58). From Cirencester, 15 ticks (5.62%, 95% CI 3.43-9.06) were PCR positive for *B. burgdorferi s.l.* along with 5 ticks (1.93%, 95% CI 0.83-4.44) from West Dean and 6 ticks (2.08%, 95% CI 0.96-4.47) from Thetford. Only one adult was positive for *B. burgdorferi s.l.* (1.52%, 95% CI 0.27-8.10). There was no significant difference in the proportions of nymphs and adults with *B. burgdorferi s.l.* (Chi-Square, $\chi^2=0.6316$, $P=0.4268$).

No ticks were found to contain *B. burgdorferi s.l.* in Wytham Woods. However, the sample sizes of each site for Wytham Woods were relatively small and so the results may not be representative given that, if the infection rates at the other locations were the same for Wytham, we could expect less than 1 infected

tick of the total 11 ticks collected there. Excluding Wytham Woods, the mean percentage of ticks harbouring *B. burgdorferi s.l.* across sites differed between Cirencester, West Dean and Thetford (Two-way ANOVA, $F_{2,7}$=9.2086, P=0.0110), however habitat had no effect (Two-way ANOVA, $F_{1,7}$=2.4653, P=0.1604). There was no interaction between location and habitat on the mean percentage of ticks with *B. burgdorferi s.l.* (Two-way ANOVA, $F_{2,7}$=2.8579, P=0.1238). The mean percentage of ticks with *B. burgdorferi s.l.* across sites was significantly greater in Cirencester than either Thetford Forest or West Dean (Tukey-Kramer, P=0.0126 and P=0.0350). There was no correlation between tick density and the proportion of ticks infected with *B. burgdorferi s.l.*. (Linear Regression, P=0.8277) and therefore locations with a high tick density cannot be predicted to have a higher proportion of infected ticks.

There were 6 ticks (4 nymphs and 2 adults) PCR positive for *B. miyamotoi* (0.73%, 95% CI 0.33-1.58; Table 2). A larger proportion of adults were infected with *B. miyamotoi* (3.03%) compared to nymphs (0.53%) (Chi-Square, χ^2=5.2604, P=0.022). One tick from Cirencester was positive for both *B. burgdorferi s.l.* and *B. miyamotoi*. The data indicates that there was no difference in the proportion of ticks containing *B. miyamotoi* between the different locations or between ecotone and woodland habitat. However, further studies using a larger sample size are required to confirm this observation.

Differentiating between *Borrelia* genospecies

Sequencing 5S-23S intergenic spacer

Of the 26 *B. burgdorferi s.l.* positive samples sequenced, 23 were successfully sequenced in the 5S-23S intergenic spacer region. The *B. burgdorferi s.s*, *B. garinii* and *B. afzelii* positive controls were sequenced successfully and no sequence was generated for the no template control. When the consensus sequences generated from the forward and reverse primers by SeqTrace (Stucky, 2012) were analysed through BLAST (Altschul et al., 1990), they were identified as 4 *B. afzelii* positives from Thetford Forest and also identified that of the remaining sequences, 12 were *B. garinii* and 7 were *B. valaisiana*.

Genospecies Identification

There were 3 samples which were not successfully sequenced. Therefore, for the statistical analysis, these samples were grouped as unconfirmed samples since the genospecies could not be identified (Table 2). The proportions of the *Borrelia burgdorferi s.l.* genospecies significantly differed between infected ticks of the different locations (Fisher's Exact Test, P=0.0025) (Fig. 3). Thetford Forest was the only location with ticks infected with *B. afzelii*. The overall percentage of ticks infected with *Borrelia* species in Cirencester was more than double that of Thetford and West Dean. Cirencester had the largest proportion of ticks infected with *B. garinii*, *B. valaisiana* and *B. miyamotoi*.

Discussion

There is comparatively little data on *B. miyamotoi* in the UK. In 2014, *B. miyamotoi* was identified in ticks from the New Forest, Salisbury and Dartmoor and it was suggested that the species had a widespread distribution (Hansford et al., 2015). However, *B. miyamotoi* was only detected in 3 nymphs through multiplex qPCR (the amplified *glpQ* and *p66* genes of one isolate were sequenced), each from 3 different locations, one of which was collected from 2009 and two from 2013 (Hansford et al., 2015). Hence it was important to confirm the presence of *B. miyamotoi*. Our data substantiate the previous findings that *B. miyamotoi* is present in *I. ricinus* in England as 6 ticks were PCR positive for *B. miyamotoi*. We report *B. miyamotoi* from ticks collected in Cirencester and the South Downs, supporting the observation of Hansford et al. (2015) that *B. miyamotoi* is widespread in Southern and South western

England but this study also extends that range to the east of England (Thetford Forest). As PCR as a technique is prone to contamination it is noteworthy that the work was performed in a laboratory that had not previously handled *B. miyamotoi* DNA providing further confidence in the results. Negative extraction controls performed during processing also remained negative.

The percentage of ticks infected with *B. miyamotoi* (0.73%) was slightly greater than the infection prevalence found by Hansford et al. (2015) (0.3%) but generally lower than that found in other European countries. Studies have reported the average percentage of *I. ricinus* ticks infected with *B. miyamotoi* to be 1.8% in Germany (Crowder et al., 2014, Eshoo et al., 2014), 3% in France (Cosson et al., 2014), 3.1% in the Netherlands (Fonville et al., 2014), 1.26% in Norway (Kjelland et al., 2015), 0.9% in Estonia (Geller et al., 2012) and 0.3-2% in Poland (Sytykiewicz et al., 2015, Kiewra et al., 2014). Previous studies have documented a 10 fold lower proportion of ticks infected with *B. miyamotoi* compared to *B. burgdorferi s.l* (Hansford et al., 2015, Barbour et al., 2009). Our study found a 4-fold difference between the two. Therefore, the risk of human *Borrelia miyamotoi* infection is likely to be lower than the risk of *Borrelia burgdorferi s.l.* infection in the UK. To date, there have been no recorded cases of *B. miyamotoi* infection in the UK, although it remains to be determined if this is because clinical cases are either not differentiated or not detected by existing diagnostic tests, or if there are no clinical infections associated with exposure to infected ticks in the UK.

Tick density differed between the different locations in our study and therefore the risk of being bitten by a tick is higher in some locations than others. Climatic factors such as temperature and relative humidity are known to effect tick distribution. High humidity has been associated with higher tick densities (Medlock et al., 2008). This is likely to be because *I. ricinus* require a relative ground humidity above 80% to avoid desiccation (Milne, 1950). However, higher humidity also enables ticks to quest for longer (Piesman and Gern, 2004). Therefore, at low humidities, there may be fewer ticks questing which are picked up by the blanket than are present in the habitat compared to areas of high humidity. Another possibility is differences in the host species population between the locations. There is a correlation between deer abundance and tick density (James et al., 2013, Ruiz-Fons and Gilbert, 2010). It is known that Thetford Forest has a notably high population of approximately 14,000 Red, Roe and Muntjac deer (Britten, 2009) which may explain why it had the highest tick density of the four locations.

Within each location, ticks were more prevalent in the ecotone between the woodland and the trail compared to within the woodland, as is consistent with previous studies (Kantsø et al., 2010). The woodland habitats sampled (with the exception of Thetford Forest Site B) had little ground vegetation, comprising mosses, pine needles and dead leaves. The ground vegetation of ecotone habitats supports a relatively high humidity and has a higher prevalence of small mammal hosts, providing a more suitable habitat for *I. ricinus* (Lindström and Jaenson, 2003).

The proportions of ticks apparently infected with the different genospecies differed between locations. Interestingly, *B. afzelii* was only found in Thetford Forest where it was more prevalent than both *B. garinii* and *B. valaisiana*. This genospecies is common in Scotland but has also been found in ticks from Southern, South-western, South-eastern and Northern England (Bettridge et al., 2013, Hansford et al., 2015, Vollmer et al., 2011). Vollmer and colleagues (2011) reported a 1.9% prevalence of *B. afzelii* in ticks from Inverness, Scotland, 1.1% in ticks from Exmoor and 0.7% in ticks from the New Forest. Contrary to this, *B. afzelii* was not present in ticks from Cirencester or the South Downs; both *B. garinii* and *B. valaisiana* dominate in these locations. Our study demonstrates the presence of *B. afzelii* in ticks from Eastern England.

The different proportions of genospecies in the different locations indicates differing host species communities. The reservoir species for *B. afzelii* are rodents and for *B. garinii* and *B. valaisiana,* the reservoir species are birds especially pheasants and songbirds (Kurtenbach et al., 1998). Since the different *Borrelia* genotypes are associated with different disease symptoms, it could be suggested that people visiting different locations should be particularly aware of certain symptoms. Infection by *B. afzelii* causes erythema migrans in 70-90% of cases however *B. garinii* only causes the symptom in 10-20% of cases (Strle and Stanek, 2009). Neuroborreliosis is also more likely during *B. garinii* infection

compared to *B. afzelii* (Strle and Stanek, 2009). Since erythema migrans is a very noticeable early symptom and neuroborreliosis is a late symptom which can be confused with other conditions, there could be more Lyme disease cases diagnosed in areas where *B. afzelii* is prominent compared to those in which *B. garnii* dominate. Unlike these two *Borrelia* species, it is still unclear if *B. valaisiana* is associated with human disease even though this species is more prevalent than *B. afzelii* in England (Bettridge et al., 2013, Vollmer et al., 2011). Although previously thought to be non-pathogenic, it has been detected in patients with erythema migrans and *B. valaisiana* DNA has been identified in the cerebrospinal fluid of a patient with probable neuroborreliosis (Rijpkema et al., 1997, Diza et al., 2004).

Of the tick life cycle stages, nymphs pose the greatest threat to humans. Although there was no difference in the proportions of adults and nymphs with *B. burgdorferi s.l.*, nymphs are involved in a greater number of tick bites than adults (Robertson et al., 2000); they are present at a higher density and are less often noticed and removed from the body compared to adults since they are smaller and located more often at the body extremities (Wilhelmsson et al., 2013). To analyse the risk to individuals of being bitten by an infected tick at each location, both tick density and the proportion of ticks infected need to be taken into account. The data show that although the tick density of Cirencester was lower than both the South Downs and Thetford, there was a significantly higher proportion of infected ticks compared with the other locations visited. The risk to individuals at each location can be calculated in terms of infected tick density, the number of infected ticks per $100m^2$ (Dobson et al., 2011). In Cirencester, there are 1.63 infected ticks per $100m^2$, in the South Downs there are 0.75 infected ticks/$100m^2$ and in Thetford Forest there are 0.9 infected ticks/$100m^2$. Therefore, there is a greater risk to people in Cirencester compared to the two "hotspots". The high number of Lyme Disease cases in "hotspots" do not provide an accurate indication of individual risk.

There are limitations to this study and it is important to note that ticks were sampled from each location only once. Tick populations fluctuate throughout the year and according to the weather (Randolph, 2004). Therefore, comparisons based on samples collected at one time in the year between the locations may not be of great use in predicting future population densities. The 2013/2014 winter was abnormally wet with higher than average rainfall and the winter and spring were also warmer than average (Met Office, 2014a, 2014b). Therefore, this could have contributed to greater tick numbers that year than previously. However, the tick densities recorded in the 4 locations are lower than those recorded for the New Forest at the same time of year in 2008 and 2009 (Dobson et al., 2011).

In conclusion, our findings provide data on questing tick density and the proportion of ticks infected with *B. burgdorferi s.l.* for locations in the UK which have not been previously tested including Thetford Forest and an area within the South Downs National Park.

Our evidence also supports the conclusions of a recent paper which first reported the presence of *B. miyamotoi* in England (Hansford et al., 2015). Further genomic analysis to examine how the UK strains of *B. miyamotoi* compare to strains circulating in other countries will be informative in understanding the biology of this organism. Identifying the presence of *B. miyamotoi* infection in humans is needed to determine if this organism poses an actual disease risk in the UK. A more comprehensive study of *B. burgdorferi s.l* and *B. miyamotoi* in tick populations should be carried out over multiple years throughout the UK. This would better inform the public and public health policy makers to the importance and risk factors associated with Lyme borreliosis and *B. miyamotoi* infection in the UK.

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Tables and Figures

Figure 1
The 4 locations sampled

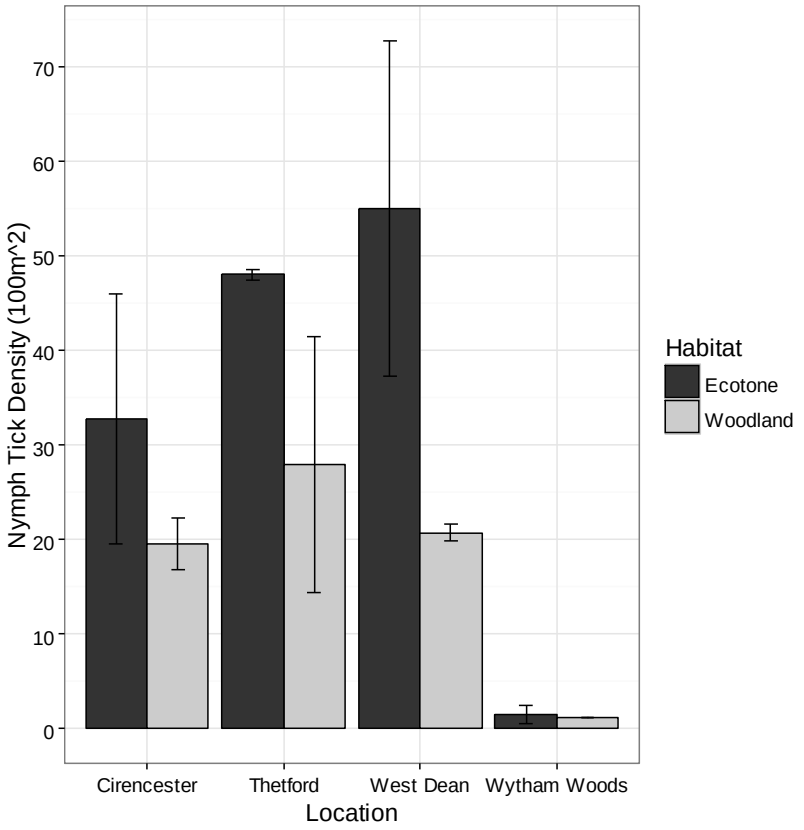
Table 1
Comparisons of numbers of ticks and tick density/100m^2 in each location

Location/Site	Nymph		Adult Male		Adult Female		Adult		Adult+Nymph	
	Number	Density	Number	Density	Number	Density	Number	Density	Number	Density
Cirencester										
Site A (E)	75	22.5	2	0.6	3	0.9	5	1.5	80	24
Site B (E)	14	16.8	1	1.2	0	0	1	1.2	15	18
Site C (W)	37	22.2	0	0	1	0.6	1	0.6	38	22.8
Site D (E)	98	58.9	4	2.4	4	2.4	8	4.8	106	63.7
Site E (W)	28	16.8	0	0	0	0	0	0	28	16.8
Total/Mean	252	27.4	7	0.8	8	0.8	15	1.6	267	29.1
West Dean										
Site A (E)	121	72.7	2	1.2	3	1.8	5	3	126	75.7
Site B (W)	36	21.6	2	1.2	0	0	2	1.2	38	22.8
Site C (E)	62	37.2	3	1.8	3	1.8	6	3.6	68	40.8
Site D (W)	33	19.8	0	0	1	0.6	1	0.6	34	20.4
Total/Mean	252	37.8	7	1.1	7	1.1	14	2.1	266	39.9
Thetford Forest										
Site A (E)	79	47.4	7	4.2	7	4.2	14	8.4	93	55.9
Site B (W)	69	41.4	6	3.6	0	0	6	3.6	75	45
Site C (E)	81	48.6	7	4.2	4	2.4	11	6.6	92	55.3
Site D (W)	24	14.4	3	1.8	1	0.6	4	2.4	28	16.8
Total/Mean	253	38	23	3.5	12	1.8	35	5.3	288	43.3
Wytham Woods										
Site A (E)	1	0.6	0	0	1	0.6	1	0.6	2	1.2
Site B (W)	2	1.2	0	0	0	0	0	0	2	1.2
Site C (E)	4	2.4	0	0	1	0.6	1	0.6	5	3
Site D (W)	2	1.2	0	0	0	0	0	0	2	1.2
Total/Mean	9	1.4	0	0	2	0.3	2	0.3	11	1.7

[1]E=Ecotone habitat, W=Woodland habitat

Fig. 2.
Comparison of mean tick densities in ecotone and woodland habitats between different locations. The bars represent 1 standard error from the mean. a., nymphs; b., adults.

a.



b.

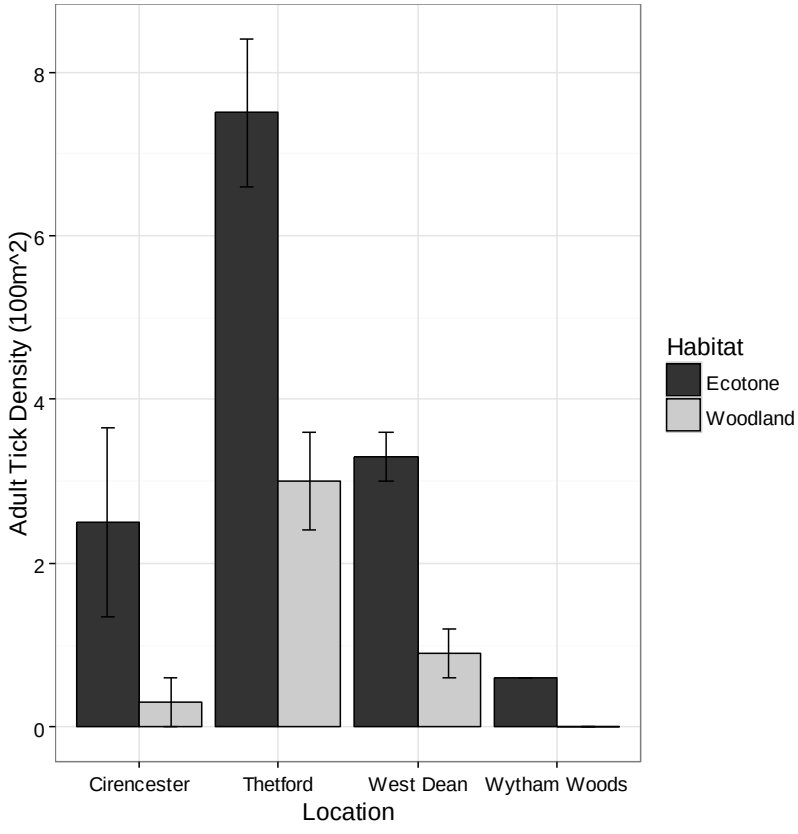


Table 2
Number and proportion (%) of PCR positive ticks collected at each location

Location	*B. afzelii*		*B. garinii*		*B. burgdorferi sensu stricto*		*B. valaisiana*		Unconfirmed genospecies		*B. miyamotoi*	
	Number	Proportion	Number	Proportion	Number	Proportion	Number	Proportion	Number	Proportion	Number	Proportion
Cirencester	0	0	8	3.00 (1.53-5.80)	0	0	6	2.25 (1.03-4.82)	1	0.38 (0.07-2.09)	3	1.12 (0.38-3.25)
West Dean	0	0	3	1.16 (0.39-3.35)	0	0	1	0.39 (0.07-2.15)	1	0.39 (0.07-2.15)	2	0.77 (0.21-2.77)
Thetford Forest	4	1.39 (0.54-3.52)[1]	1	0.35 (0.06-1.94)	0	0	0	0	1	0.35 (0.06-1.94)	1	0.35 (0.06-1.94)
Wytham Woods	0	0	0	0	0	0	0	0	0	0	0	0

[1]95% confidence interval

Fig. 3.
Proportion of ticks containing the different *B. burgdorferi* genospecies and *B. miyamotoi* in each location